Heme and Chlorophyll Intake and Risk of Colorectal Cancer in the Netherlands Cohort Study

Helena F. Balder,[1] Johan de Vogel,[2] Margje C.J.F. Jansen,[1] Matty P. Weijenberg,[3] Piet A. van den Brandt,[3] Susanne Westenbrink,[1] Roelof van der Meer,[2] and R. Alexandra Goldbohm[1]

[1]Department Food and Chemical Risk Analysis, TNO Quality of Life, Zeist, the Netherlands; [2]Wageningen Centre for Food Sciences and NIZO Food Research, Department of Health and Safety, Ede, the Netherlands; and [3]Nutrition and Toxicology Research Institute Maastricht, Department of Epidemiology, Maastricht University, Maastricht, the Netherlands

Abstract

Background: **The evidence for red meat as a determinant of colorectal cancer remains equivocal, which might be explained by differences in heme content. Heme is the pro-oxidant, iron-containing porphyrin pigment of meat and its content depends on the type of meat. Chlorophyll from green vegetables might modify this association.**
Methods: **The Netherlands Cohort Study was initiated in 1986 when a self-administered questionnaire on risk factors for cancer was completed by 120,852 subjects ages 55 to 69 years. After 9.3 years of follow-up through the Cancer Registry, 1,535 incident colorectal cancer cases (869 men and 666 women) were available. Nineteen of the 150 items in the validated dietary questionnaire related to consumption of specific types of fresh and processed meat. Heme iron content was calculated as a type-specific percentage of the total iron content and chlorophyll content of vegetables was derived from the literature.**
Results: **Multivariate rate ratios for quintiles of heme iron intake and colon cancer were 1.00, 0.98, 1.04, 1.13, and 1.29 (P_{trend} = 0.10) among men and 1.00, 1.31, 1.44, 1.18, and 1.20 (P_{trend} = 0.56) among women, respectively. No consistent associations were observed for rectal cancer. Rate ratios for colon cancer increased across successive quintiles of the ratio of heme/chlorophyll among men only (1.00, 1.08, 1.01, 1.32, and 1.43; P_{trend} = 0.01). No associations were observed between fresh meat and colorectal cancer.**
Conclusion: **Our data suggest an elevated risk of colon cancer in men with increasing intake of heme iron and decreasing intake of chlorophyll. Further research is needed to confirm these results.** (Cancer Epidemiol Biomarkers Prev 2006;15(4):717–25)

Introduction

Results from cohort studies suggest that consumption of (fresh) ''red'' meat is modestly associated with the risk of colon cancer (1-3). However, results differ between studies and over time. In some, no association was observed (4), whereas in others a strong association was seen (5, 6). Recent results from seven cohort studies (7-13) only partly confirm a modest association with red meat (including processed meat in most cases; refs. 9-12). Virtually, all cohort studies found an increased risk for colon cancer with processed meat consumption (2, 7-12). Results for rectal cancer were inconsistent.

An explanation for the inconsistent and relatively modest associations might be that not total red meat intake, defined by many authors as beef, pork, and lamb, but heme increases risk. Heme is the iron porphyrin component of hemoproteins, such as hemoglobin and myoglobin, and gives meat its red color. These proteins are digested in the upper gastrointestinal tract, releasing heme in the gut lumen (14). Heme is a pro-oxidant that has been shown previously to increase colonic epithelial proliferation and toxicity of fecal water in rats (15). These effects were specific for heme because equimolar dietary ferric citrate and protoporphyrin did not increase proliferation and cytotoxicity (15). Dietary heme was metabolized in the gut lumen and resulted in the formation of a highly cytotoxic factor that damaged the colonic mucosa (16). This resulted in a compensatory hyperproliferation of the epithelium, which may increase the risk of colon cancer (17). In addition, Pierre et al. (18) showed that red meat and heme increased formation of aberrant crypt foci in rats. A role for heme in colorectal cancer etiology is further supported by Bingham et al. (19, 20). They showed in randomized crossover experiments in a metabolic suite that male volunteers, exposed to high amounts of red meat or heme, produced higher levels of fecal *N*-nitroso compounds than when exposed to the same amounts of white meat or ferrous iron.

Previous results from the Netherlands Cohort Study on Diet and Cancer (NLCS) indicated that fresh meat consumption as such was not a risk factor for colon cancer (4). However, intake of iron was found to be a risk factor in men (21). Lee et al. (22) reported previously that the relative risks for (proximal) colon cancer increased >2-fold across categories of heme iron intake in the Iowa Women's Health Study, especially among women who drink alcohol. Larsson et al. (23) also observed an increased risk for colon cancer with a high heme iron intake in a female Swedish cohort, but the association was equally likely as that for meat as such. To date, heme intake is usually assessed by applying a fixed factor to the total iron content of all meat items regardless of the origin of the meat. However, it is apparent from the literature that not only the absolute total iron content differs substantially between meat from different origins but also the percentage iron from heme, which is high in beef and low in poultry and fish (24). To assess heme intake more accurately, we took the origin of the meat (beef, pork, chicken, etc.) into account by estimating the heme content of specific types of meat based on published literature.

In addition to a hypothesized positive association between heme intake and colorectal cancer risk, we hypothesized that chlorophyll, the ubiquitous pigment in green vegetables,

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Requests for reprints: R. Alexandra Goldbohm, Department Food and Chemical Risk Analysis, TNO Quality of Life, P.O. Box 360, 3700 AJ Zeist, the Netherlands. Phone: 31-30-694-4750; Fax: 31-30-69-44070. E-mail: bausch@voeding.tno.nl

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modifies this association. Chlorophyll is structurally similar to heme, as it contains a porphyrin ring similar to that of heme but with a central, nonreactive magnesium instead of iron atom. Chlorophyll may block the reactivity of heme in the gastrointestinal tract and thus prevent the formation of cytotoxic heme metabolites. We showed previously in rats that addition of spinach or purified chlorophyll to a heme diet inhibited heme metabolism in the gut and prevented the heme-induced formation of a cytotoxic factor that increased colonic cytotoxicity and epithelial proliferation (16).

The aim of this article was to determine the association of heme in combination with chlorophyll intake with risk of colorectal cancer in the NLCS, a population-based prospective cohort study with 9.3 years of follow-up. To our knowledge, this has not previously been studied in an observational setting.

Subjects and Methods

Study Population. The study design of the NLCS has been reported in detail elsewhere (25). The NLCS was initiated in 1986 when a self-administered questionnaire on dietary habits, lifestyle characteristics, medical history, and other potential risk factors for cancer was completed by 58,279 men and 62,573 women ages 55 to 69 years. After baseline exposure measurement, a subcohort of 5,000 subjects was randomly sampled from the large cohort. Following the case-cohort approach, this subcohort was followed up for vital status and migration to estimate person time at risk accumulated in the cohort. The cohort-at-large has been followed-up for incident cancer by record linkage to the Netherlands Cancer Registry and the Netherlands Pathology Registry (PALGA) for 9.3 years (26).

After excluding subjects who reported prevalent cancer other than skin cancer at baseline and subjects with incomplete or inconsistent dietary information, 869 male and 666 female cases with primary colon (*International Classification of Diseases for Oncology* 153.0-153.9) or rectal cancer (*International Classification of Diseases for Oncology* 154.0 and 154.1) and 2,156 male and 2,215 female subcohort members were available for analysis.

The NLCS has been approved by the institutional review boards of the TNO Nutrition and Food Research Institute (Zeist) and Maastricht University (Maastricht).

Questionnaire. The dietary part of this questionnaire consisted of a 150-item semiquantitative food frequency questionnaire (FFQ) on the usual intake of food and beverages in the year preceding the start of the study, which was validated against a 9-day diet record (27). The Spearman correlation coefficients for fresh meat, processed meat (meat products), fish, and vegetables were 0.46, 0.54, 0.53, and 0.38 respectively. As the classification of foods into food groups did not entirely overlap, due to differences in the coding of recipes between the FFQ and record, the observed correlation between the food groups was likely to be lower than the true. On average, the FFQ captured between 85% and 98% of the absolute intake of energy, animal protein, and total (fresh) meat assessed by the record but only 57% of processed meat.

Questionnaire data of all cases and subcohort members have been key-entered twice and blinded with respect to case/subcohort status to avoid random and systematic coding errors.

Assessment of Heme Iron Intake. The FFQ contained 14 items on consumption of meat with the hot meal (mainly fresh meat, including chicken), 5 items on consumption of processed meat (meat products) used as sandwich filling, and 3 items on fish consumption. To derive an individual serving size of fresh meat, a question was included on the quantity of meat usually purchased (per person, per meal). For chicken and fish, standard serving sizes were used. Fresh meat was defined as meat that has not undergone some form of preservation and includes beef, pork, minced meat, chicken, liver, and other meat (i.e., horse and lamb). Processed meat was defined as meat items that have undergone some form of preservation [i.e., smoking, fermentation, and/or treatment with nitrate and/or nitrite salt (''curing'')]. Meat items in the questionnaire were converted into mean daily consumption in grams.

We decided to use the total iron content of each heme-containing food item from the Dutch Food Composition Database (28), which was used to calculate nutrient intake in the NLCS, as a starting point, as this takes into account the specific types, cuts, and fat composition of the Dutch food items. To investigate the levels of heme for different types of meat, poultry, and fish, a literature search was done. The results were used to derive the mean percentage heme iron relative to total iron for each specific origin of the meat (beef, pork, chicken, fish, etc.). We selected only those studies that measured total iron directly and, after lipid extraction, heme iron in the same meat sample (24, 29-32). The average percentages were 65, 39, and 26 for cooked beef, pork, and chicken or fish, respectively. Multiplying the type-specific percentages of heme iron with the total iron content (mg/g) yielded heme iron contents for all heme-containing food items in the NLCS database. Individual mean daily intake of heme iron was assessed by multiplying the estimated heme iron content with the mean daily intake of the relevant food items.

Assessment of Chlorophyll Intake. With regard to vegetable consumption, participants were asked to report their frequency of consumption of several vegetables both in summer and in winter. Usual serving sizes were asked for string beans and cooked endive only, the mean of which served as an indicator for serving sizes of all cooked vegetables (33). Standard serving sizes were used for lettuce and other raw vegetables. For tomatoes and sweet peppers, consumption was asked in pieces per week and month, respectively, during summer and winter. We used the derived individual portion sizes to convert these frequencies and amounts to consumption in grams per day. Items included in the questionnaire covered almost all vegetables and fruits eaten regularly at baseline measurement.

To assess chlorophyll intake, publications reporting chlorophyll content in vegetables were searched. However, this information seemed to be scarce in published literature. Khachik et al. (34) reported total chlorophyll contents for broccoli, cabbage, spinach, brussels sprouts, and kale. We classified all vegetables from the NLCS FFQ into five categories most resembling these five vegetables analyzed by Khachik et al. Throughout the classification of the vegetables, we also took the level of green coloring, assumed to correlate with chlorophyll content, and the shape of the vegetables into account, as vegetables growing on a head usually have a core that is pale compared with the outer leaves. The five vegetable categories were not green colored (white, yellow, red, etc.) vegetables (0 mg/100 g), pale green vegetables with a light core (leek and broad beans, 2 mg/100 g), green vegetables (brussels sprouts, green cabbage, green beans, and green peppers, 6 mg/100 g), green leafy vegetables (endive, spinach, and lettuce, 130 mg/100 g), and dark green leafy vegetables (kale, 185 mg/100 g).

To check our classification of the vegetables included in our questionnaire but not analyzed by Khachik et al., we also measured the chlorophyll content of some of these vegetables relative to that of spinach, which was used as reference. We extracted chlorophyll from freeze-dried endive, lettuce, tomato, and spinach (bought in a local supermarket) by washing them with 80% acetone until colorless (35). The concentration chlorophyll in the extracts of these vegetables was determined by comparing their absorption spectra with those of standard chlorophyll *a* and *b* solutions in 80% acetone (Sigma-Aldrich, St. Louis, MO) on a spectrophotometer (Lambda 2; Perkin-Elmer, Norwalk, CT). Our classification of the vegetables concerned in the five chlorophyll categories was in agreement with their classification based on the chemical analysis. Mean daily intake of chlorophyll was calculated by multiplying the estimated chlorophyll content of the vegetables with their respective intake.

Statistical Analyses. All analyses were conducted separately for men and women. Cox proportional hazards models were constructed to estimate hazard ratios and 95% confidence intervals (95% CI) relating intake of heme iron and chlorophyll to the incidence of colorectal, colon, and rectal cancer (Stata version 9, Stata Corp., College Station, TX). The proportional hazards assumption was tested using Schoenfeld residuals. Because of the case-cohort design, the 95% CIs were corrected for the additional variance introduced by using a random

Table 1. Mean (SD) daily intake of heme iron (mg/d) according to categories of nondietary and dietary characteristics of participants of the NLCS

	Men				Women			
	Subcohort		Colorectal cancer cases		Subcohort		Colorectal cancer cases	
	n (%)	Heme iron (mg/d)	*n* (%)	Heme iron (mg/d)	*n* (%)	Heme iron (mg/d)	*n* (%)	Heme iron (mg/d)
All	2,156 (100)	1.16 (0.51)	869 (100)	1.17 (0.53)	2,215 (100)	0.98 (0.48)	666 (100)	0.97 (0.43)
Age (y)								
55-59	834 (39)	1.19 (0.51)	244 (28)	1.25 (0.62)	869 (39)	0.98 (0.46)	165 (25)	0.99 (0.44)
60-64	746 (35)	1.16 (0.51)	325 (37)	1.14 (0.46)	738 (33)	0.95 (0.45)	254 (38)	0.94 (0.40)
65-69	576 (27)	1.13 (0.52)	300 (35)	1.14 (0.53)	608 (27)	0.99 (0.55)	247 (37)	0.97 (0.44)
BMI (kg/m^2)								
<23	425 (20)	1.06 (0.50)	149 (17)	1.07 (0.54)	597 (27)	0.90 (0.48)	154 (23)	0.88 (0.44)
23-24.9	689 (32)	1.16 (0.51)	262 (30)	1.20 (0.55)	600 (27)	0.95 (0.48)	174 (26)	0.96 (0.37)
25-26.9	565 (26)	1.18 (0.49)	231 (27)	1.13 (0.51)	418 (19)	0.99 (0.45)	138 (21)	0.98 (0.45)
≥27	404 (19)	1.23 (0.56)	195 (22)	1.28 (0.52)	525 (24)	1.06 (0.49)	173 (26)	1.04 (0.44)
Family history of colorectal cancer								
No	2,044 (95)	1.16 (0.52)	783 (90)	1.18 (0.54)	2,092 (94)	0.98 (0.49)	609 (91)	0.96 (0.42)
Yes	112 (5)	1.11 (0.41)	86 (10)	1.16 (0.51)	123 (6)	0.97 (0.45)	57 (9)	1.00 (0.47)
Cigarette smoking status								
Never	272 (13)	1.12 (0.53)	90 (10)	1.15 (0.51)	1,287 (58)	0.98 (0.49)	406 (61)	0.96 (0.41)
Former	1,105 (51)	1.14 (0.49)	521 (60)	1.15 (0.52)	459 (21)	0.96 (0.46)	139 (21)	0.97 (0.42)
Current	779 (36)	1.21 (0.53)	258 (30)	1.23 (0.58)	469 (21)	0.98 (0.49)	121 (18)	0.99 (0.49)
Nonoccupational physical activity (min/d)								
<30	392 (18)	1.15 (0.55)	136 (16)	1.20 (0.56)	547 (25)	0.97 (0.51)	199 (30)	0.98 (0.46)
30-60	661 (31)	1.15 (0.50)	268 (31)	1.14 (0.53)	679 (31)	0.96 (0.45)	193 (29)	0.97 (0.40)
60-90	401 (19)	1.15 (0.48)	177 (20)	1.19 (0.53)	491 (22)	0.97 (0.46)	149 (22)	0.93 (0.41)
>90	678 (31)	1.18 (0.52)	277 (32)	1.20 (0.53)	468 (21)	1.03 (0.52)	117 (18)	1.00 (0.44)
Total energy intake, MJ/d (median)								
Q1 (m/w, 6.5/5.1)*	430 (20)	0.94 (0.45)	183 (21)	0.98 (0.46)	442 (20)	0.82 (0.42)	141 (21)	0.85 (0.45)
Q2 (m/w, 7.9/6.1)	431 (20)	1.07 (0.48)	175 (20)	1.10 (0.50)	444 (20)	0.92 (0.48)	141 (21)	0.90 (0.42)
Q3 (m/w, 8.9/6.9)	431 (20)	1.16 (0.48)	200 (23)	1.15 (0.47)	443 (20)	0.96 (0.42)	138 (21)	0.96 (0.37)
Q4 (m/w, 10/7.8)	432 (20)	1.21 (0.47)	159 (18)	1.24 (0.53)	445 (20)	1.00 (0.51)	131 (20)	1.06 (0.39)
Q5 (m/w, 12/9.2)	432 (20)	1.42 (0.55)	152 (17)	1.45 (0.63)	441 (20)	1.17 (0.51)	115 (17)	1.09 (0.46)
Alcohol intake (g/d)								
0	303 (14)	1.06 (0.50)	111 (13)	1.04 (0.62)	681 (31)	0.95 (0.54)	229 (34)	0.94 (0.44)
0.1-4.9	447 (21)	1.13 (0.52)	199 (23)	1.15 (0.54)	765 (34)	0.96 (0.48)	206 (31)	0.93 (0.43)
5-14.9	585 (27)	1.13 (0.50)	190 (22)	1.17 (0.50)	394 (18)	1.00 (0.40)	120 (18)	1.00 (0.41)
15-29.9	480 (22)	1.22 (0.49)	202 (23)	1.19 (0.46)	201 (9)	1.04 (0.45)	57 (9)	1.11 (0.40)
≥30	313 (15)	1.27 (0.53)	162 (19)	1.30 (0.58)	73 (3)	1.07 (0.48)	29 (4)	1.09 (0.31)
Total vegetable consumption, g/d (median)								
Q1 (m/w, 102/106)*	432 (20)	1.05 (0.47)	183 (21)	1.08 (0.49)	446 (20)	0.86 (0.38)	147 (22)	0.93 (0.40)
Q2 (m/w, 144/149)	431 (20)	1.11 (0.44)	160 (18)	1.12 (0.50)	443 (20)	0.96 (0.44)	129 (19)	0.92 (0.44)
Q3 (m/w, 177/183)	435 (20)	1.17 (0.51)	181 (21)	1.15 (0.48)	449 (20)	0.96 (0.44)	129 (19)	0.95 (0.41)
Q4 (m/w, 218/224)	432 (20)	1.21 (0.53)	174 (20)	1.20 (0.51)	439 (20)	1.03 (0.50)	135 (20)	1.04 (0.42)
Q5 (m/w, 295/300)	426 (20)	1.26 (0.58)	171 (20)	1.34 (0.64)	438 (20)	1.07 (0.61)	126 (19)	0.99 (0.46)
Total fresh meat intake,† g/d (median)								
Q1 (m/w, 56/45)*	425 (20)	0.75 (0.38)	188 (22)	0.76 (0.38)	443 (20)	0.55 (0.30)	114 (17)	0.50 (0.28)
Q2 (m/w, 86/74)	429 (20)	0.98 (0.31)	173 (20)	1.04 (0.38)	441 (20)	0.83 (0.30)	129 (19)	0.86 (0.28)
Q3 (m/w, 103/91)	441 (20)	1.12 (0.38)	181 (21)	1.21 (0.48)	438 (20)	1.00 (0.37)	157 (24)	1.01 (0.35)
Q4 (m/w, 124/108)	428 (20)	1.29 (0.41)	172 (20)	1.27 (0.43)	448 (20)	1.10 (0.37)	137 (21)	1.10 (0.36)
Q5 (m/w, 158/146)	433 (20)	1.66 (0.54)	155 (18)	1.68 (0.56)	445 (20)	1.39 (0.58)	129 (19)	1.28 (0.43)
Total processed meat intake, g/d (median)								
0 (0)	201 (9)	0.81 (0.46)	78 (9)	0.85 (0.51)	296 (13)	0.67 (0.43)	87 (13)	0.67 (0.37)
0.1-9.9 (5)	684 (32)	0.98 (0.41)	277 (32)	1.00 (0.43)	987 (45)	0.86 (0.40)	295 (44)	0.88 (0.40)
10-19.9 (14)	610 (28)	1.14 (0.43)	239 (28)	1.15 (0.44)	539 (24)	1.06 (0.43)	169 (25)	1.03 (0.35)
≥20 (32)	661 (31)	1.47 (0.53)	275 (32)	1.46 (0.58)	393 (18)	1.38 (0.51)	115 (17)	1.31 (0.38)
Total iron intake, mg/d (median)								
Q1 (m/w, 9.5/8.5)*	430 (20)	0.85 (0.33)	178 (20)	0.88 (0.34)	443 (20)	0.70 (0.31)	144 (22)	0.71 (0.31)
Q2 (m/w, 11/10)	433 (20)	1.01 (0.38)	158 (18)	1.00 (0.33)	442 (20)	0.85 (0.34)	145 (22)	0.89 (0.37)
Q3 (m/w, 13/11)	429 (20)	1.15 (0.43)	191 (22)	1.12 (0.40)	441 (20)	0.94 (0.37)	127 (19)	0.95 (0.32)
Q4 (m/w, 15/13)	435 (20)	1.28 (0.51)	159 (18)	1.28 (0.52)	446 (20)	1.01 (0.41)	137 (21)	1.10 (0.43)
Q5 (m/w, 17/15)	429 (20)	1.52 (0.59)	183 (21)	1.58 (0.68)	443 (20)	1.38 (0.64)	113 (17)	1.24 (0.50)

*Median values for men/women (m/w).
†Based on raw weight.

Table 2. RRs (95% CIs) of colorectal, colon, and rectal cancer according to quintiles of heme iron intake of men and women in the NLCS (9.3 years of follow-up)

	Men						
	Q1	Q2	Q3	Q4	Q5	P_{trend}	Continuous*
Median heme intake (mg/d)	0.60	0.87	1.08	1.34	1.85		
Person-years	3,716	3,767	3,709	3,779	3,718		18,688
Colorectal cancer							
Cancer cases (n)	167	164	190	179	169		869†
Age-adjusted RR (95% CI)	1 (Reference)	0.99 (0.76-1.28)	1.16 (0.90-1.49)	1.08 (0.84-1.39)	1.06 (0.82-1.37)	0.46	1.07 (0.91-1.24)
Multivariate RR‡ (95% CI)	1 (Reference)	0.99 (0.75-1.29)	1.13 (0.86-1.48)	1.06 (0.80-1.39)	1.16 (0.87-1.55)	0.27	1.11 (0.93-1.33)
>2 y RR§ (95% CI)	1 (Reference)	1.11 (0.83-1.49)	1.20 (0.90-1.61)	1.20 (0.89-1.62)	1.32 (0.96-1.80)	0.08	1.15 (0.95-1.39)
Colon cancer							
Cancer cases (n)	108	100	109	114	108		539
Age-adjusted RR (95% CI)	1 (Reference)	0.94 (0.69-1.27)	1.03 (0.76-1.39)	1.07 (0.80-1.45)	1.06 (0.78-1.43)	0.47	1.04 (0.86-1.25)
Multivariate RR‡ (95% CI)	1 (Reference)	0.98 (0.71-1.35)	1.04 (0.75-1.44)	1.13 (0.82-1.56)	1.29 (0.92-1.81)	0.10	1.16 (0.94-1.43)
>2 y RR§ (95% CI)	1 (Reference)	1.08 (0.76-1.53)	1.07 (0.75-1.53)	1.29 (0.90-1.83)	1.50 (1.03-2.17)	0.02	1.22 (0.98-1.52)
Rectal cancer							
Cancer cases (n)	59	64	82	66	62		333
Age-adjusted RR (95% CI)	1 (Reference)	1.08 (0.74-1.58)	1.41 (0.98-2.01)	1.10 (0.76-1.60)	1.08 (0.74-1.58)	0.69	1.11 (0.89-1.39)
Multivariate RR‡ (95% CI)	1 (Reference)	1.00 (0.68-1.49)	1.30 (0.89-1.90)	0.97 (0.65-1.45)	0.98 (0.64-1.50)	0.84	1.04 (0.80-1.35)
>2 y RR§ (95% CI)	1 (Reference)	1.17 (0.75-1.80)	1.44 (0.95-2.19)	1.10 (0.71-1.72)	1.06 (0.66-1.71)	0.94	1.04 (0.79-1.38)

*Per increment of 1 mg heme/d.
†The no. colorectal cancer cases is lower than no. colon plus rectal cancer cases due to cases with tumors in both sites.
‡Adjusted for age at baseline (y), BMI (kg/m^2), family history of colorectal cancer (yes, no), cigarette smoker (never, former, current), nonoccupational physical activity (<30, 30-60, 60-90, >90 min/d), total energy intake (kJ), consumption of alcohol (0-4.9, 5-14.9, 15-29.9, ≥30 g/d for men; 0, 0.1-4.9, 5-14.9, ≥15 g/d for women), and total vegetable consumption (g/d).
§Cases diagnosed during the first 2 years of follow-up were excluded.

sampled subcohort instead of the complete cohort by using the robust option. Rate ratios (RR) for heme iron were estimated for quintiles based on the sex-specific distribution in the subcohort and as a continuous variable with an increment of 1 mg/d. Two-sided tests for trend in the RRs were assessed by fitting ordinal exposure categories as continuous variables. To evaluate whether early symptoms of disease before diagnosis could have influenced the results, early cases (diagnosed within 2 years after baseline) were also excluded from the analyses.

Age at baseline, education, cigarette smoking, nonoccupational physical activity, body mass index (BMI), intake of energy, alcohol, folate, and fiber and total vegetable consumption were considered as potential confounders based on their association with risk of colorectal cancer. Of these, age at baseline (years), cigarette smoking status (never, former, current), nonoccupational physical activity (<30, 30-60, 60-90, >90 min/d), BMI (continuous, kg/m^2), alcohol intake (gender-specific categories), and total vegetable consumption (continuous, g/d) were included in the confounder-adjusted models as they were associated with heme intake and seemed to affect the RR estimates. Family history of colorectal cancer in first- or second-degree relatives, a strong determinant of colorectal cancer risk, was added to the multivariate models to reduce residual variation.

To evaluate the combined effect of heme and chlorophyll on the risk of colorectal, colon, and rectal cancer, indicator variables were included in the regression model, representing tertiles of heme iron by tertiles of the intake of chlorophyll, using the lowest tertile of heme and the highest tertile of chlorophyll as the reference category. Total vegetable consumption was analyzed in the same manner. Presence of effect

Table 3. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer according to total iron intake, total fresh meat and processed meat consumption, and chlorophyll intake of men and women in the NLCS (9.3 years of follow-up)

	Men					
	Q1	Q2	Q3	Q4	Q5	P_{trend}
Colorectal cancer						
Total iron	1 (Reference)	0.92 (0.69-1.23)	1.16 (0.86-1.55)	1.02 (0.75-1.39)	1.34 (0.93-1.93)	0.12
Fresh meat	1 (Reference)	1.00 (0.77-1.30)	0.91 (0.70-1.19)	0.93 (0.72-1.22)	0.82 (0.62-1.08)	0.15
Processed meat (categories)	1 (Reference)	1.02 (0.74-1.41)	0.98 (0.71-1.36)	1.18 (0.84-1.64)		0.25
Chlorophyll	1 (Reference)	0.94 (0.72-1.22)	0.88 (0.68-1.15)	0.87 (0.66-1.13)	0.94 (0.72-1.23)	0.50
Colon cancer						
Total iron	1 (Reference)	0.90 (0.64-1.26)	0.98 (0.69-1.40)	0.85 (0.58-1.24)	1.30 (0.84-2.01)	0.43
Total fresh meat	1 (Reference)	1.01 (0.74-1.38)	1.07 (0.79-1.47)	1.04 (0.76-1.43)	0.88 (0.63-1.23)	0.59
Processed meat (categories)	1 (Reference)	1.11 (0.75-1.63)	1.09 (0.73-1.63)	1.33 (0.89-1.99)		0.13
Chlorophyll	1 (Reference)	0.88 (0.65-1.20)	0.73 (0.53-1.00)	0.78 (0.57-1.06)	0.78 (0.57-1.07)	0.08
Rectal cancer						
Total iron	1 (Reference)	1.02 (0.67-1.56)	1.54 (1.02-2.32)	1.39 (0.89-2.16)	1.44 (0.85-2.45)	0.08
Total fresh meat	1 (Reference)	1.03 (0.72-1.48)	0.73 (0.49-1.07)	0.81 (0.56-1.18)	0.78 (0.53-1.14)	0.09
Processed meat (categories)	1 (Reference)	0.90 (0.58-1.40)	0.83 (0.52-1.31)	0.96 (0.60-1.53)		0.99
Chlorophyll	1 (Reference)	1.19 (0.74-1.89)	1.45 (0.92-2.29)	1.08 (0.67-1.75)	1.27 (0.80-2.02)	0.49

NOTE: Adjusted for age at baseline (y), BMI (kg/m^2), family history of colorectal cancer (yes, no), cigarette smoker (never, former, current), nonoccupational physical activity (<30, 30-60, 60-90, >90 min/d), total energy intake (kJ), consumption of alcohol (0-4.9, 5-14.9, 15-29.9, ≥30 g/d for men; 0, 0.1-4.9, 5-14.9, ≥15 g/d for women), and total vegetable consumption (g/d).

Table 2. RRs (95% CIs) of colorectal, colon, and rectal cancer according to quintiles of heme iron intake of men and women in the NLCS (9.3 years of follow-up) (Cont'd)

Women						
Q1	Q2	Q3	Q4	Q5	P_{trend}	Continuous*
0.47	0.71	0.92	1.13	1.54		
4,038	4,028	3,965	3,991	4,013		20,035
119	133	149	137	128		666
1 (Reference)	1.12 (0.84-1.48)	1.29 (0.98-1.70)	1.16 (0.88-1.54)	1.11 (0.83-1.47)	0.46	0.96 (0.81-1.13)
1 (Reference)	1.14 (0.84-1.54)	1.39 (1.03-1.87)	1.19 (0.87-1.62)	1.22 (0.89-1.68)	0.22	1.00 (0.83-1.21)
1 (Reference)	1.09 (0.78-1.51)	1.47 (1.08-2.02)	1.18 (0.85-1.65)	1.20 (0.86-1.69)	0.24	0.99 (0.81-1.20)
84	108	108	96	88		484
1 (Reference)	1.29 (0.94-1.77)	1.32 (0.97-1.81)	1.15 (0.84-1.59)	1.08 (0.78-1.50)	0.94	0.90 (0.74-1.09)
1 (Reference)	1.31 (0.94-1.85)	1.44 (1.02-2.01)	1.18 (0.83-1.68)	1.20 (0.83-1.74)	0.56	0.96 (0.77-1.20)
1 (Reference)	1.26 (0.87-1.83)	1.56 (1.09-2.23)	1.26 (0.86-1.83)	1.19 (0.80-1.77)	0.43	0.97 (0.78-1.22)
35	26	41	43	40		185
1 (Reference)	0.73 (0.43-1.23)	1.15 (0.72-1.83)	1.23 (0.77-1.95)	1.17 (0.73-1.88)	0.14	1.12 (0.88-1.43)
1 (Reference)	0.72 (0.40-1.27)	1.19 (0.72-1.98)	1.27 (0.76-2.13)	1.23 (0.73-2.07)	0.11	1.12 (0.86-1.46)
1 (Reference)	0.69 (0.37-1.29)	1.18 (0.68-2.05)	1.07 (0.60-1.91)	1.20 (0.68-2.12)	0.25	1.05 (0.76-1.43)

modification (on the multiplicative scale) was tested using the interaction term of the continuous heme and chlorophyll variables. Biological interaction was assessed by comparing the sum of the main effects with the combined effect (36). In addition, risk of colorectal cancer was estimated for quintiles of the molar ratio of heme/chlorophyll. Subjects with a high score on an error index for measurement of vegetable consumption, which was available in the data set (33), were excluded from this analysis to avoid unrealistic high and low ratios.

Colon cancer was also divided in proximal cancer (cecum through transverse colon; *International Classification of Diseases for Oncology* codes 153.0,153.1, 153.4, 153.5, and 153.6) and distal cancer (splenic flexure through sigmoid colon; *International Classification of Diseases for Oncology* codes 153.2, 153.3, and 153.7) for the analyses of main effects of heme, chlorophyll, and the heme/chlorophyll ratio.

Modification of the heme-colorectal cancer association by alcohol consumption was assessed by dividing subjects in three (gender-specific) categories of alcohol consumption.

Results

A description of the 869 male and 666 female colorectal cancer cases and 2,156 male and 2,215 female subcohort members is presented in Table 1. On average, cases were older, more overweight, and more likely to report a family history of colorectal cancer than members of the subcohort. Heme intake was higher among men compared with women. The correlation of heme iron intake and fresh meat and processed meat were 0.65 and 0.51, respectively, for men and 0.63 and 0.50, respectively, for women. In addition, heme intake was positively associated with BMI, total energy and alcohol intake, and total vegetable consumption in both genders.

Table 2 presents RRs for heme intake and colorectal cancer estimated in a Cox proportional hazards model. After adjustment for confounders, there was an indication of a consistent positive but not statistically significant association between heme iron intake and risk of colorectal and colon cancer in men, which became more evident after exclusion of cases diagnosed in the first 2 years of follow-up (Table 2). The RR for colon cancer and the continuous heme variable was 1.16 (95% CI, 0.94-1.43) per increment of heme intake of 1 mg/d. After additional adjustment for fresh and processed meat, the RR increased to 1.38 (95% CI, 0.99-1.92) per increment of 1 mg heme/d.

No association was observed for male rectal cancer or for both cancer sites among women. Results for proximal and distal colon cancer showed that the associations were not confined to a particular site (data not shown).

Table 3. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer according to total iron intake, total fresh meat and processed meat consumption, and chlorophyll intake of men and women in the NLCS (9.3 years of follow-up) (Cont'd)

Women					
Q1	Q2	Q3	Q4	Q5	P_{trend}
1 (Reference)	1.22 (0.91-1.63)	1.01 (0.74-1.39)	1.11 (0.80-1.55)	1.08 (0.72-1.62)	0.90
1 (Reference)	1.12 (0.82-1.52)	1.30 (0.97-1.75)	1.13 (0.83-1.53)	1.10 (0.80-1.51)	0.57
1 (Reference)	1.04 (0.78-1.39)	1.13 (0.82-1.55)	1.05 (0.74-1.48)		0.62
1 (Reference)	0.80 (0.59-1.08)	1.11 (0.84-1.48)	0.97 (0.73-1.30)	0.91 (0.68-1.23)	0.99
1 (Reference)	1.30 (0.94-1.81)	0.89 (0.62-1.29)	1.12 (0.77-1.64)	1.14 (0.73-1.80)	0.91
1 (Reference)	1.04 (0.73-1.47)	1.30 (0.94-1.80)	1.07 (0.76-1.51)	1.00 (0.70-1.45)	0.89
1 (Reference)	1.00 (0.72-1.39)	1.06 (0.74-1.51)	1.07 (0.73-1.57)		0.61
1 (Reference)	0.75 (0.54-1.05)	1.06 (0.78-1.46)	0.87 (0.63-1.20)	0.81 (0.58-1.13)	0.46
1 (Reference)	0.97 (0.57-1.66)	1.41 (0.83-2.38)	1.13 (0.63-2.04)	1.11 (0.53-2.30)	0.63
1 (Reference)	1.42 (0.83-2.43)	1.30 (0.76-2.22)	1.37 (0.80-2.35)	1.40 (0.81-2.42)	0.33
1 (Reference)	1.19 (0.71-2.02)	1.42 (0.81-2.50)	1.01 (0.54-1.90)		0.83
1 (Reference)	0.99 (0.52-1.89)	1.41 (0.77-2.58)	1.48 (0.81-2.70)	1.27 (0.68-2.35)	0.19

Table 4. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer by tertiles of heme iron intake according to tertiles of dietary intake of chlorophyll, total vegetable consumption, consumption frequency of green leafy vegetables, and alcohol consumption among men

	Heme iron, mg/d (median)		
	Colorectal cancer		
	Tertile 1 (0.67)	Tertile 2 (1.08)	Tertile 3 (1.74)
Chlorophyll, mg/d (median intake)			
Tertile 3 (86)	1 (Reference)	1.17 (0.79-1.73)	1.14 (0.78-1.68)
Tertile 2 (48)	1.21 (0.82-1.77)	1.13 (0.77-1.67)	1.19 (0.80-1.78)
Tertile 1 (26)	1.13 (0.77-1.65)	1.31 (0.90-1.92)	1.34 (0.90-1.99)
Vegetables, g/d (median intake)			
Tertile 3 (283)	1 (Reference)	1.41 (0.96-2.07)	1.28 (0.88-1.87)
Tertile 2 (178)	1.35 (0.92-1.99)	1.16 (0.78-1.72)	1.24 (0.83-1.85)
Tertile 1 (113)	1.16 (0.79-1.71)	1.25 (0.85-1.84)	1.37 (0.90-2.07)
Alcohol consumption, g/d (median)*			
0-4.9 (1.4)	1 (Reference)	1.06 (0.76-1.49)	1.06 (0.74-1.50)
5-14.9 (10)	0.75 (0.51-1.09)	0.80 (0.55-1.16)	1.07 (0.72-1.59)
≥15 (31)	1.15 (0.81-1.63)	1.24 (0.90-1.70)	1.13 (0.82-1.56)

NOTE: Adjusted for age at baseline (y), BMI (kg/m^2), family history of colorectal cancer (yes, no), cigarette smoker (never, former, current), nonoccupational physical activity (<30, 30-60, 60-90, >90 min/d), total energy intake (kJ), and consumption of alcohol (except for analysis of alcohol). Green leafy vegetables include spinach, endive (cooked and raw), kale, and lettuce.
*Additional adjustment for total vegetable consumption (g/d).

Table 3 presents RRs for chlorophyll intake and for heme-related variables that may alternatively explain the relation between heme intake and colon cancer. The highest compared with the lowest quintile of chlorophyll intake was inversely but not statistically significant associated with risk of colon cancer in men (RR, 0.78; 95% CI, 0.57-1.07, $P\text{s}_{\text{trend}}$ = 0.08). This association was less consistent in women ($P\text{s}_{\text{trend}}$ = 0.46). Whereas among men no association was observed between total iron and fresh meat and colon cancer, a small increased risk was seen for men eating >20 g processed meat/d (RR, 1.33; 95% CI, 0.89-1.99). The association of processed meat with colon cancer attenuated when heme iron intake was included in the model (RR for the highest compared with the lowest quintile of processed meat consumption was 1.23; 95% CI, 0.80-1.89 for men; data not shown).

The combined effect of heme and chlorophyll intake was evaluated by calculating the risk of colorectal cancer for heme in tertiles of chlorophyll intake (Tables 4 and 5). The category that we hypothesized to be associated with the lowest risk (low heme in combination with high chlorophyll) was used as the reference category. High intake of heme in combination with low intake of chlorophyll seemed to be associated with an elevated risk of colon cancer in men (RR, 1.58; 95% CI, 0.99-2.54). There was no evidence of effect modification on the multiplicative scale ($P\text{s}_{\text{interaction}}$ = 0.40) and weak evidence for biological interaction assessed on the additive scale. Similar results were observed for the combined effect of heme and vegetables, although the dose-response association was less consistent. The hypothesized highest risk category corresponded with a RR of 2.02 (95% CI, 1.22-3.34) compared with the lowest risk category. Neither for male rectal cancer nor for female colon or rectal cancer an association emerged in the hypothesized direction. We also estimated the heme colon cancer risk for the molar ratio of heme/chlorophyll. Multivariate RRs (95% CIs) for successive quintiles of the heme/chlorophyll ratio compared with the lowest quintile were 1.08 (0.77-1.51), 1.01 (0.72-1.41), 1.32

Table 5. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer by tertiles of heme iron intake according to tertiles of dietary intake of chlorophyll, total vegetable consumption, consumption frequency of green leafy vegetables, and alcohol consumption among women

	Heme iron, mg/d (median)		
	Colorectal cancer		
	Tertile 1 (0.53)	Tertile 2 (0.91)	Tertile 3 (1.47)
Chlorophyll, mg/d (median intake)			
Tertile 3 (84)	1 (Reference)	1.13 (0.73-1.75)	1.21 (0.80-1.84)
Tertile 2 (48)	1.08 (0.71-1.66)	1.32 (0.88-2.00)	1.43 (0.94-2.19)
Tertile 1 (25)	1.12 (0.74-1.70)	1.23 (0.81-1.86)	1.10 (0.71-1.69)
Vegetables, g/d (median intake)			
Tertile 3 (284)	1 (Reference)	0.94 (0.61-1.43)	1.11 (0.74-1.66)
Tertile 2 (184)	0.78 (0.51-1.19)	1.17 (0.78-1.75)	0.97 (0.64-1.48)
Tertile 1 (117)	0.97 (0.65-1.45)	1.02 (0.68-1.53)	1.08 (0.71-1.65)
Alcohol consumption, g/d (median)*			
0	1 (Reference)	1.65 (1.13-2.42)	1.24 (0.83-1.86)
0.1-4.9 (1.9)	1.11 (0.75-1.64)	1.02 (0.69-1.52)	1.08 (0.72-1.64)
≥5 (17)	1.18 (0.76-1.81)	1.15 (0.76-1.73)	1.48 (1.00-2.19)

NOTE: Adjusted for age at baseline (y), BMI (kg/m^2), family history of colorectal cancer (yes, no), cigarette smoker (never, former, current), nonoccupational physical activity (<30, 30-60, 60-90, >90 min/d), total energy intake (kJ), and consumption of alcohol (except for analysis of alcohol). Green leafy vegetables include spinach, endive (cooked and raw), kale, and lettuce.
*Additional adjustment for total vegetable consumption (g/d).

Table 4. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer by tertiles of heme iron intake according to tertiles of dietary intake of chlorophyll, total vegetable consumption, consumption frequency of green leafy vegetables, and alcohol consumption among men (Cont'd)

Heme iron, mg/d (median)					
Colon cancer			Rectal cancer		
Tertile 1 (0.67)	Tertile 2 (1.08)	Tertile 3 (1.74)	Tertile 1 (0.67)	Tertile 2 (1.08)	Tertile 3 (1.74)
1 (Reference)	1.06 (0.65-1.71)	1.16 (0.73-1.85)	1 (Reference)	1.45 (0.85-2.47)	1.12 (0.65-1.93)
1.14 (0.72-1.82)	1.09 (0.68-1.75)	1.30 (0.80-2.09)	1.32 (0.76-2.26)	1.20 (0.69-2.07)	1.02 (0.57-1.82)
1.27 (0.81-1.99)	1.30 (0.82-2.06)	1.58 (0.99-2.54)	0.87 (0.49-1.55)	1.37 (0.80-2.34)	0.98 (0.54-1.76)
1 (Reference)	1.50 (0.92-2.44)	1.47 (0.91-2.36)	1 (Reference)	1.30 (0.78-2.16)	1.01 (0.60-1.69)
1.70 (1.05-2.73)	1.39 (0.85-2.26)	1.46 (0.89-2.4))	0.88 (0.51-1.50)	0.89 (0.52-1.54)	0.95 (0.55-1.64)
1.39 (0.86-2.24)	1.26 (0.77-2.06)	2.02 (1.22-3.34)	0.83 (0.48-1.43)	1.20 (0.71-2.01)	0.63 (0.34-1.18)
1 (Reference)	0.90 (0.60-1.34)	1.03 (0.68-1.55)	1 (Reference)	1.48 (0.90-2.43)	1.17 (0.69-2.00)
0.68 (0.43-1.06)	0.77 (0.50-1.18)	1.13 (0.72-1.79)	0.96 (0.55-1.68)	1.01 (0.58-1.75)	0.95 (0.50-1.77)
0.99 (0.65-1.50)	1.01 (0.69-1.48)	1.06 (0.73-1.54)	1.50 (0.91-2.48)	1.77 (1.11-2.82)	1.30 (0.79-2.13)

(0.95-1.84), and 1.43 (1.03-1.97) in men (Ps_{trend} = 0.01; Fig. 1) and 1.33 (0.94-1.87), 1.15 (0.81-1.64), 1.34 (0.95-1.89), and 1.12 (0.78-1.59) in women (Ps_{trend} = 0.61). As no statistically significant effect modification by sex existed (P = 0.38), we also determined the results for the heme/chlorophyll ratio for both sexes combined. The multivariate RRs (95% CIs) for successive quintiles of the heme/chlorophyll ratio compared with the lowest quintile were 1.20 (0.95-1.51), 0.98 (0.77-1.24), 1.20 (0.95-1.52), and 1.29 (1.03-1.63; Ps_{trend} = 0.05). These results were very similar for proximal and distal colon cancer (data not shown).

Tables 4 and 5 also address the association of heme and colorectal cancer across three levels of alcohol consumption. In men, no evidence of a differential association was observed. In women, a small elevated risk of colon and rectal cancer was observed for those in the highest tertile of heme intake and drinking >5 g alcohol/d compared with nondrinkers with a low heme intake (RR for colorectal cancer, 1.48; 95% CI, 1.00-2.19).

Discussion

In this cohort study with 539 male and 448 female incident colon cancer patients and 333 male and 185 female incident rectal cancer patients, a small positive association was observed between heme intake and colon cancer among men but not among women. After stratification by chlorophyll intake, the male subjects with the lowest chlorophyll and the highest heme intake had the highest risk of colon cancer and this was also shown by the statistically significant positive association between the heme/chlorophyll ratio and colon cancer in men. No consistent associations were observed for rectal cancer in both men and women.

The prospective design and the completeness of follow-up of our study (>95%; ref. 37) ensured that information bias due to disease status and selection bias due to loss of follow-up are unlikely. In addition, the associations did not seem to be spuriously produced by symptoms of early but not yet diagnosed disease, as the exclusion of cases diagnosed within the first 2 years of follow-up made the associations more evident, indicating that early cases had a somewhat lower heme intake than later cases.

Our study also has some limitations. Measurement error can be substantial in dietary assessment. The validation study of our FFQ has shown that it does relatively well (27), but measurement error will still have attenuated associations. Furthermore, as neither heme nor chlorophyll was included in the Dutch food composition table, the content of these compounds was estimated based on reported levels in the literature. This may have resulted in additional measurement error, although the estimation of heme intake was based on

Table 5. Multivariate-adjusted RRs (95% CIs) of colorectal, colon, and rectal cancer by tertiles of heme iron intake according to tertiles of dietary intake of chlorophyll, total vegetable consumption, consumption frequency of green leafy vegetables, and alcohol consumption among women (Cont'd)

Heme iron, mg/d (median)					
Colon cancer			Rectal cancer		
Tertile 1 (0.53)	Tertile 2 (0.91)	Tertile 3 (1.47)	Tertile 1 (0.53)	Tertile 2 (0.91)	Tertile 3 (1.47)
1 (Reference)	0.99 (0.61-1.60)	0.96 (0.60-1.54)	1 (Reference)	1.65 (0.73-3.76)	2.23 (1.04-4.79)
0.98 (0.61-1.57)	1.24 (0.79-1.96)	1.27 (0.79-2.04)	1.51 (0.68-3.36)	1.55 (0.70-3.44)	2.11 (0.97-4.60)
1.07 (0.67-1.69)	1.21 (0.77-1.92)	1.04 (0.64-1.68)	1.40 (0.63-3.11)	1.17 (0.52-2.66)	1.40 (0.61-3.21)
1 (Reference)	0.92 (0.58-1.48)	0.96 (0.61-1.53)	1 (Reference)	0.86 (0.40-1.85)	1.43 (0.73-2.77)
0.75 (0.46-1.21)	1.15 (0.73-1.81)	0.92 (0.58-1.48)	0.80 (0.38-1.70)	1.08 (0.54-2.16)	1.07 (0.53-2.18)
1.01 (0.65-1.58)	1.04 (0.66-1.64)	1.04 (0.65-1.69)	0.75 (0.36-1.57)	0.81 (0.39-1.68)	1.13 (0.55-2.32)
1 (Reference)	1.91 (1.24-2.95)	1.26 (0.79-2.01)	1 (Reference)	0.93 (0.47-1.83)	1.20 (0.63-2.30)
1.36 (0.88-2.10)	1.02 (0.64-1.61)	1.10 (0.68-1.77)	0.52 (0.24-1.12)	0.99 (0.51-1.90)	1.02 (0.52-2.03)
1.24 (0.76-2.04)	1.26 (0.79-2.00)	1.50 (0.95-2.36)	1.05 (0.51-2.15)	0.82 (0.40-1.68)	1.41 (0.76-2.61)

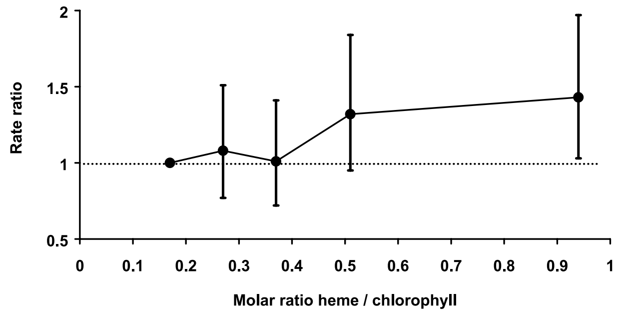

Figure 1. RRs and 95% CIs of colon cancer in men according to quintiles of the molar ratio of heme/chlorophyll intake ($P_{trend} = 0.01$). *Points,* RRs; *bars,* 95% CIs.

more available data than that of chlorophyll intake. However, although there is a considerable chance of misclassification, it is unlikely that this misclassification would be differential with regard to the end point. In addition, heme iron content values as calculated in this study (i.e., based on type-specific percentage of total iron content) seemed to be in reasonable agreement with absolute heme iron values in meat and fish available from the same literature sources. Finally, although our results for heme support prior biological hypotheses and agree with studies in rats (15) and humans (20), we cannot rule out the possibility of chance findings within small subgroups.

Previously published results on meat and colorectal cancer risk in the NLCS, based on 3.3 years (4) and 7.3 years (21) of follow-up, showed no association between consumption of total fresh meat and fish and colon cancer risk in men and women. A positive association for both men and women was initially observed for processed meat and colon cancer (4) but diminished, particularly in women, after longer follow-up. The current results for fresh meat and colon cancer, based on extended follow-up, are still in line with the previous.

The weak but consistent association between heme intake and colon cancer observed in men was driven by consumption of (cooked) beef, which contains three to four times as much heme as (cooked) pork. The contribution of each type of meat to the total fresh meat consumption was ~40%, 42%, 14%, and 4% for beef, pork, chicken, and other meat (including horse and lamb), respectively, in this population. The relative high proportion of pork may explain the absence of an association with fresh meat in our study. Other cohorts have frequently used ''red meat,'' which includes beef, pork, and lamb, as key variable in meat and cancer studies but have only rarely reported on the association between colorectal cancer and specific types of meat also because some frequently used FFQs did not distinguish beef from pork consumption. In North American cohorts, consumption of beef is roughly thrice higher than that of pork. However, recent results from the European Prospective Investigation into Cancer and Nutrition showed a stronger association of colorectal cancer risk with pork than with beef plus veal (12).

The RRs for heme and colon cancer became stronger when additional adjustment for fresh and processed meat was done, indicating that a meat effect, if any, is more likely to be due to heme and not to other constituents or preparation methods of meat. Most epidemiologic studies that determined the risk of specific constituents of (red) meat considered only total dietary iron (38). However, as ~90% of the total dietary iron consists of non-heme iron, associations of total dietary iron with colorectal cancer in most epidemiologic studies may be attenuated by other compounds contained in the nonmeat sources of this non-heme iron. Two studies examined associations among colon cancer incidence and dietary intake of heme iron among women (22, 23). Both observed an increased risk of colon cancer with increasing heme iron intake; however, this association was confined to proximal colon tumors in the Iowa Women's Health Study (22), whereas the study in Sweden likely observed the association for distal tumors only (11). In both studies, the positive association between heme iron and colon cancer was stronger among women who consumed alcohol than among those who consumed little or no alcohol. Neither study took the type of meat into account in the assessment of heme iron as they applied a fixed percentage (40%) to the total iron content of all meat items. Although we did not observe an association between heme intake and colon cancer among women, we observed a suggestion of an association with colon and rectal cancer among women who drank >5 g alcohol (0-1 glass)/d, which is comparable with the level above which an effect was seen in Iowa and Sweden (22, 23).

We did not beforehand anticipate a different association for men and women. However, there are several plausible explanations for the different findings. An explanation could be the total intake of heme and chlorophyll. Men are known to consume more food in general and more meat and relatively less vegetable than women as was also evident from our data. Alternatively, it could also be hypothesized that, as women need more iron due to menstrual losses and as heme iron is more easily absorbed compared with non-heme iron, relatively more iron from heme is absorbed in women, so that less heme is available during lifetime up to menopause to form the cytotoxic factor in the bowel.

Our results are also consistent with those of experimental studies among human volunteers (19, 20). As these studies were conducted in men only, we do not know whether women would have shown a different effect of red meat and heme.

Evidence of a protective effect of (green) vegetables for colorectal cancer risk (39, 40) stimulated us to investigate the interaction of heme and spinach in an animal model study. We showed that spinach or an equimolar amount of chlorophyll inhibited dietary heme-induced luminal cytotoxicity and damage to colonic mucosa in rats (16). The addition of chlorophyll to a heme diet prevented the formation of a cytotoxic heme metabolite. We speculated that chlorophyll traps heme in hydrophobic heme-chlorophyll complexes in the gut lumen and as a result blocks the pro-oxidant activity of heme (16, 41). This mechanism implies that heme and chlorophyll must be consumed simultaneously. The present study indicates that an increase in risk is indeed associated with an increase in the heme iron to chlorophyll ratio, although the evidence for biological interaction between heme and chlorophyll was not strong. However, our FFQ was not designed to capture combinations of specific types of meat with specific types of vegetables in the same meal, although vegetables are mostly eaten in combination with meat, particularly in this population with traditional dietary habits. We can therefore expect that biological interaction, if present, is underestimated due to dilution effects of the heme and chlorophyll variables calculated as mean daily intake. A human experimental study has not observed inhibition of heme-induced formation of *N*-nitroso compounds by vegetables, but the vegetables used in that study (broccoli, peas, and brussels sprouts) were low in chlorophyll compared with high levels of heme in the type and quantities of meat used (42). Our rat studies showed only detrimental dietary heme-induced effects in the absence or at low concentrations of chlorophyll.[4] Furthermore, processed meats, in contrast

[4] In preparation.

to fresh meats, are often not consumed together with vegetables. This might also explain why the association with processed meats and colorectal cancer is clearer than for fresh meats.

In conclusion, we hypothesized that heme might be positively associated with colon cancer risk despite absence of an association with meat and that chlorophyll intake would modify this association. Our results confirm these hypotheses in part, but further research is needed, particularly on the role of chlorophyll and the association in women.

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